Published online July 27, 2017

Journal of Environmental Quality SHORT COMMUNICATIONS

Plastic Biofilm Carrier after Corn Cobs Reduces Nitrate Loading in Laboratory Denitrifying Bioreactors

Gary W. Feyereisen,* Laura E. Christianson, Thomas B. Moorman, Rodney T. Venterea, and Jeffrey A. Coulter

Abstract

Nitrate-nitrogen (nitrate-N) removal rates can be increased substantially in denitrifying bioreactors with a corn (*Zea mays* L.) cob bed medium compared with woodchips; however, additional organic carbon (C) is released into the effluent. This laboratory column experiment was conducted to test the performance of a postbed chamber of inert plastic biofilm carrier (PBC) after corn cobs (CC) to extend the area of biofilm colonization, enhance nitrate-N removal, lower total organic C losses, and reduce nitrous oxide (N_2O) production at warm (15.5°C) and cold (1.5°C) temperatures. Treatments were CC only and CC plus PBC in series (CC-PBC). Across the two temperatures, nitrate-N load removal was 21% greater with CC-PBC than CC, with 54 and 44% of total nitrate N load, respectively. However, total organic C concentrations and loads were not significantly different between treatments. Colonization of the PBC by denitrifiers occurred, although gene abundance at the outlet (PBC) was less than at the inlet (CC). The PBC chamber increased nitrate-N removal rate and reduced cumulative N_2O production at 15.5°C, but not at 1.5°C. Across temperatures and treatments, N_2O production was 0.9% of nitrate-N removed. Including an additional chamber filled with PBC downstream from the CC bioreactor provided benefits in terms nitrate-N removal but did not achieve C removal. The presence of excess C, as well as available nitrate, in the PBC chamber suggests another unidentified limiting factor for nitrate removal.

Core Ideas

- A chamber of inert plastic biofilm carrier after corn cobs increased NO_3-N removal.
- The added chamber did not achieve aqueous carbon removal.
- Denitrifiers colonized the plastic biofilm carrier.
- At 15.5°C, nitrous oxide cumulative production decreased, but not at 1.5°C.
- Cold temperature performance was unaffected by the added chamber.

J. Environ. Qual. 46:915–920 (2017)
doi:10.2134/jeq2017.02.0060
Supplemental material is available online for this article.
Received 6 Feb. 2017.
Accepted 20 Apr. 2017.
*Corresponding author (gary.feyereisen@ars.usda.gov).

BIOLOGICAL nitrogen (N) removal from wastewater using heterotrophic denitrification requires the presence of denitrifying bacteria, anoxic conditions, an electron donor (e.g., a C-based substrate), and a terminal electron acceptor (nitrate). In wastewater treatment for nitrate removal, moving beds packed with plastic biofilm carriers (PBCs) provide surface area for colonization and growth of denitrifying bacteria within the water matrix, and a soluble C source (e.g., methanol) is typically added to fuel the denitrification process (Tchobanoglous et al., 2003). A denitrifying bioreactor for the treatment of nonregulated agricultural tile drainage is a simple modification of this process involving the use of a solid organic C medium (e.g., woodchips or corn [*Zea mays* L.] cobs [CC]) that serves as both a colonization surface area and a C source.

Within a woodchip denitrifying bioreactor, the wood surface is important for attachment and growth of microorganisms that are involved with the release of available C and with denitrification, but there is a general consensus that wood particle size (e.g., fine vs. coarse woodchips) has little impact on overall nitrate removal (Greenan et al., 2006; van Driel et al., 2006). This may be due to the permeable nature of these biological materials, where microsites within the media provide additional surface area for reactions. For example, Robertson (2010) observed dark rings (i.e., "dark brown reaction rims"), an indication of biological activity, extending several millimeters into woodchips after denitrification column tests.

It is well established that wood media in denitrifying bioreactors release organic nutrient loads, especially under startup conditions (Schipper et al., 2010), which is a concern because of the role of organic C in freshwater ecology (Stanley et al., 2012). This surplus C is unused for heterotrophic denitrification. Organic C losses in bioreactor effluent are exacerbated in bioreactors

G.W. Feyereisen and R.T. Venterea, USDA-ARS-Soil & Water Management Research Unit, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108; L.E. Christianson, Dep. of Crop Sciences, Univ. of Illinois, Urbana, IL 61801; T.B. Moorman, USDA-ARS-National Laboratory for Agriculture and the Environment, Ames, IA 50011; J.A. Coulter, Dep. of Agronomy & Plant Genetics, Univ. of Minnesota, St. Paul, MN 55455. Use of manufacturers' names does not represent endorsement by the USDA. The USDA is an equal opportunity employer. Assigned to Associate Editor Zach Easton.

Abbreviations: CC, corn cobs; CC-PBC, bioreactor treatment with corn cobs chamber followed by plastic biofilm carrier chamber; *cp*N_2O, cumulative nitrous oxide production; *d*N_2O, dissolved nitrous oxide; HRT, hydraulic residence time; NRR, nitrate-nitrogen removal rate on the basis of corn cob chamber volume alone; $NRR_{CC\text{-}PBC}$, nitrate-nitrogen removal rate based on corn cob and plastic biofilm chamber volumes together; ORP, oxidation reduction potential; PBC, plastic biofilm carrier; *p*N_2O, nitrous oxide production rate; PVC, polyvinyl chloride; qPCR, quantitative polymerase chain reaction; *r*N_2O, cumulative nitrous oxide production relative to cumulative removal of nitrate (%); TC, total carbon; TOC, total organic carbon.

filled with agricultural residues, although these residues support greater nitrate removal rates than woodchips (Cameron and Schipper, 2010; Feyereisen et al., 2016). Although it is generally thought that nitrate removal is not limited by surface area of the solid organic C media, incorporation of inert material into a bioreactor for additional surface area may provide biological treatment of leached organics and enhance water treatment.

Several researchers have investigated or alluded to use of a biofilm carrier to enhance denitrification. Andersson et al. (2008) identified biofilm carriers that enhanced denitrification of municipal wastewater by providing surfaces for biofilm attachment. Other researchers have used PBCs as support media for denitrification in agricultural (Cantafio et al., 1996) and aquacultural (Saliling et al., 2007) applications using methanol as a C source. A PBC has been found to support denitrification over a range of temperatures (3–20°C) in a laboratory-scale batch reactor (Welander and Mattiasson, 2003). Elgood et al. (2010) suggested that a polishing module consisting of inert media be added at the outlet of a bioreactor to further consume methane, which they measured in their study. Ibrahim et al. (2015) simultaneously treated N and phosphorus in columns containing cells of woodchips followed by zeolite and pea-sized gravel. To date, the use of an inert PBC downstream from a carbonaceous-bed denitrifying bioreactor to effect additional nitrate-N and aqueous C removal via denitrification has not been demonstrated.

The findings and suggestions of previous researchers concerning the potential low-temperature improvement in denitrification of a PBC (Welander and Mattiasson, 2003) and the need for a postbed polishing module (Elgood et al., 2010) contributed to the ideas tested in this study. Therefore, the study was conducted to investigate performance of a novel design wherein a noncarbonaceous, plastic medium was placed in series after an organic bioreactor medium to provide additional surface area to support denitrifying bacteria. We hypothesized that use of the plastic medium would (i) provide area for biofilm development, (ii) reduce nitrate-N load, (iii) lessen aqueous C in effluent, (iv) decrease dissolved N_2O in effluent, and (v) improve performance at near-freezing temperatures.

Materials and Methods

Media Treatments, Bioreactor Design, and Operation

Two treatments, CC and CC-PBC, were evaluated using triplicate columns of each treatment. The CC treatment columns consisted of a chamber filled with CC media, and the CC-PBC treatment columns consisted of a chamber filled with the same CC medium but followed by an additional section of chamber containing PBC material. A synthetic water solution of relatively high nitrate-N concentration (50 mg N L^{-1}), chosen to ensure that nitrate-N removal rates (NRR) were not nitrate limited, was pumped upward through the columns at a flow rate yielding a hydraulic residence time (HRT) through the CC chambers of 12 h for 130 d at 15.5°C. Drainable porosities (24 h) were determined prior to the experiment and used to calculate HRT as drainable porosity times gross chamber volume divided by flow rate. Fresh media were repacked, and the experiment was repeated at 1.5°C. The temperatures were representative of summer and spring snowmelt conditions in the US northern Corn Belt (Supplemental Fig. S1).

Specifically, CC were soaked in tap water, inoculated with a Waukegan silt loam soil (fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls) (Greenan et al., 2009) with a history of manure application, and packed in eight 400-g layers in polyvinyl chloride (PVC) columns (15.2 cm in diameter × 49.5 cm long)(Supplemental Fig. S2a). To the three CC-PBC replicates, a PVC coupling was glued to the top of the column and an additional length of pipe was glued into the coupling, extending the height of the column by 25 cm (Supplemental Fig. S2b). A PBC (Kaldnes K3, Veolia Water Technologies AB-AnoxKaldnes) was dampened with tap water, mixed with the silt loam, and packed into the column extension on top of the CC. Silt loam addition to both treatments totaled 140 ± 0.5 dry g $column^{-1}$; for CC-PBC the soil was split between the CC and PBC chambers, 126 ± 0.5 and 14 ± 0.5 dry g $column^{-1}$, respectively. Individual plastic carrier pieces are 25-mm-diameter × 10-mm-long open cylinders with a webbed cross-section of high surface area (specific surface area: 500 m^2 m^{-3}; Tchobanoglous et al., 2003). The packed density of the media in the experiment was 0.11 g cm^{-3}.

Initially, reverse-osmosis water was circulated to flush detritus, identify and fix leaks, and calibrate the pump. At completion of the 15.5°C run, the caps were removed, the columns were emptied, and both media were sampled for microbial analysis at the inlets and outlets of the columns. The columns were repacked with fresh materials, and the experiment was repeated at 1.5°C, after which media sampling was reperformed. After the 1.5°C experiment, for the CC-PBC treatment, CC were sampled at the outlet end of the CC chamber. Additional specifics of the experimental setup are in Feyereisen et al. (2016). Data for the CC treatment reported in this paper were reported in Feyereisen et al. (2016).

Sample Collection and Analyses

Water Sampling and Analysis

Water samples were collected and analyzed as described in Feyereisen et al. (2016). Briefly, thrice-weekly samples (125 mL) were taken, acidified, stored at 4°C, and analyzed by cadmium-reduction colorimetry for nitrate-N (NO_3–N + NO_2–N) and by ultraviolet light and persulfate oxidation with infrared detection (EPA Methods 415.1 and 9060A) for total C (TC) and total organic C (TOC) obtained with acid pretreatment. Nitrate-N load removal was calculated as the difference in cumulative nitrate load into and out of each column for the duration of each of the temperature experiments. For each sampling date, NRR, with units of mg N m^{-3} d^{-1}, was calculated by dividing nitrate-N mass removal by the time between samplings and normalizing by gross volume of the CC chamber. For CC-PBC, NRR was also calculated by normalizing by the sum of gross volumes of the CC and PBC chambers ($NRR_{CC\text{-}PBC}$). Net TC and net TOC loads were calculated by summing the differences of TC and TOC mass in and mass out of each column for each sampling period.

Every 4 wk, additional water sampling was conducted for analysis of dissolved N_2O (dN_2O). Samples (12 mL) were collected by syringe and injected into glass 40-mL vials preserved with 0.1 mL H_2SO_4. The vials were equilibrated at 23°C for 1 h, and headspace gas was then transferred by syringe to 9-mL glass vials and analyzed for N_2O by gas chromatography (Agilent/

Hewlett-Packard) (Venterea et al., 2010). The dN$_2$O was determined using Henry's Law coefficient (Sander, 2015). Samples were collected on Days 2, 30, 49, 77, 105, and 133 at 15.5°C and on Days 14, 42, 70, 98, and 114 at 1.5°C. The N$_2$O production rate (pN$_2$O) was calculated analogously to NRR. Cumulative N$_2$O production (cpN$_2$O) was determined by trapezoidal integration of pN$_2$O versus time over a common time period (Days 14–114) at both temperatures. Cumulative N$_2$O production relative to cumulative removal of nitrate-N (rN$_2$O) was determined over this 100-d period and expressed as a percentage.

Molecular Analysis of Microbial Populations

Media samples were collected and frozen after each experiment. Quantitative polymerase chain reaction (qPCR) assays targeting the *nosZ* gene clade 1, *nosZ* gene clade 2, and 16S ribosomal RNA genes were performed as described in Feyereisen et al. (2016) to quantify bacteria carrying the N$_2$O reductase gene. The Power Max Soil Kit (MoBio) was used to extract DNA from 2 to 5 g dry mass of PBC or CC. Each qPCR assay included standards (known quantities of target gene DNA) and negative controls. Each sample result is the mean of three qPCR reactions, expressed as gene copy number g^{-1} on a dry-mass basis. Preparation of standards and details of the thermocycling are described in Feyereisen et al. (2016).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 2011) at $P \leq 0.10$. Temperature and treatment were considered fixed effects, and block and interactions with block were considered random effects. Blocks represent replications, since column replicates were grouped by distance from the chamber door. Sampling date was considered a categorical variable, and sampling date and position within bioreactor were considered fixed effects and repeated measurements. Time-series data were analyzed separately by temperature run (15.5 and 1.5°C) due to differences in sampling dates. Abundance of *nosZ* (clade 1 and clade 2) and the 16S ribosomal RNA genes, expressed as gene copies g^{-1} material on a dry-mass basis and as gene copies cm^{-3}, were logarithm base 10 transformed prior to statistical analysis to meet the assumptions of normality and common variance. Gene abundance data were subjected to a three-factor analysis of temperature, treatment, and position within bioreactor as main effects. An additional two-factor analysis of treatment and position within bioreactor (inlet vs. outlet end of CC) was conducted for gene abundance data from samples collected from the experiment conducted at 1.5°C. Means were compared with independent pairwise t-tests at $P \leq 0.10$ using the PDIFF option of the MIXED procedure of SAS.

Results

Hydrology and Hydraulic Residence Time

The HRTs for the CC column sections were similar for the CC and CC-PBC treatments both for the 15.5 and 1.5°C experiments—11.8 and 11.5 h and 13.5 and 13.6 h, respectively (P = 0.55 and 0.95, respectively; Supplemental Table S1). The additional column sections containing PBC increased HRT (P = 0.006); HRTs for the entire CC-PBC columns were 24.5 and 26.6 h for the 15.5 and 1.5°C runs, respectively. Thus, HRT in the PBC chambers was similar to that in the CC chambers of the CC-PBC and CC treatments.

Nitrate-N Load Reductions, Nitrate-N Concentrations, and Nitrate Removal Rate

When averaged across temperatures, CC-PBC removed 53.7% of nitrate-N entering the columns, significantly greater load removal than the 44.5% for CC (P = 0.032, Table 1). Across treatments, cumulative nitrate-N load removal was greater at 15.5 than 1.5°C, 79.6 versus 18.6%, respectively (P = 0.002). The temperature × treatment interaction was insignificant (P = 0.21) for cumulative nitrate-N load removal. The main effect of treatment on effluent nitrate-N concentration was significantly different for the 15.5°C run, but not for the 1.5°C run (P = 0.065 and 0.170, respectively). The treatment × day interaction for effluent nitrate-N concentration was significant (P < 0.001, Supplemental Table S2) at 15.5°C, but not at 1.5°C (P = 0.68). At 15.5°C, effluent nitrate-N concentration for CC-PBC was less than for CC on 23 of 48 dates (Fig. 1, Supplemental Table S2).

The NRR for CC-PBC at 15.5°C was significantly greater than for CC (P = 0.099), ranging from 30.6 to 57.8 (mean 40.2 g N m^{-3}d^{-1}) and from 23.7 to 46.5 (mean 34.9 g N m^{-3}d^{-1}), respectively (Table 1). At 15.5°C, NRRs for CC-PBC exceeded those for CC on 18 of 48 sampling dates (Supplemental Table S3). At 1.5°C, NRRs were not significantly different for CC-PBC and CC (P = 0.143), although NRRs for CC-PBC exceeded those for CC on 37 of 53 sampling dates (Supplemental Table S4); values ranged from 7.7 to 14.5 (mean 9.7 g N m^{-3}d^{-1}) and from 5.8 to 11.4 (mean 7.4 g N m^{-3} d^{-1}), respectively. The treatment × day interaction was significant for NRR for both 15.5 and 1.5°C (P = 0.006 and < 0.001, respectively). Nitrate removal rate based on total CC plus PBC compartment volume (NRR$_{CC\text{-}PBC}$) was greater for CC than CC-PBC at 15.5°C (P = 0.022, Table 1), but not at 1.5°C (P = 0.37). The treatment × day interaction for NRR$_{CC\text{-}PBC}$ was significant both at 15.5 and 1.5°C (P = 0.070 and P < 0.001, respectively). At 15.5°C, NRR$_{CC\text{-}PBC}$ was greater for CC than CC-PBC on 44 of 48 sampling dates (Supplemental Table S5), and at 1.5°C, NRR$_{CC\text{-}PBC}$ was greater for CC than CC-PBC on 3 of 53 sampling dates (Supplemental Table S6).

Carbon Loads and Concentrations

Net cumulative TC and TOC loads were not significantly different between treatments for either temperature experiment (Table 1). Concentration of TOC was not significantly different between treatments for either temperature experiment (Fig. 2). There was a significant treatment × day interaction for TC concentration at 1.5°C (P = 0.047); TC concentration was greater for CC-PBC than for CC on 2 of 53 sampling dates (Supplemental Table S7).

Oxidation Reduction Potential and pH

Oxidation reduction potential (ORP) was not affected by the PBC, with sampling means ranging from −89 to 225 mV and 9 to 201 mV at 15.5°C for CC and CC-PBC, respectively, and from 241 to 404 and 239 to 386 mV at 1.5°C for CC and CC-PBC, respectively. At 1.5°C, the treatment × day interaction was significant for ORP (P = 0.004); ORP for CC-PBC was less than for CC on 2 of 53 sampling dates. There was no significant difference in pH between treatments for either temperature experiment, nor were treatment × day interactions significant; pH ranged narrowly from 6.87 to 7.62 and 5.72 to 7.63 at 15.5°C

Table 1. Nitrate-nitrogen (nitrate-N) removal rates, nitrate-N load reductions as a percentage of the cumulative inlet nitrate-N load averaged across days, temperature sensitivity (Q_{10}), cumulative total carbon and total organic carbon loads, cumulative nitrous oxide production (cpN_2O), ratio of nitrous oxide produced to nitrate-N removed (rN_2O), and microbial abundance for the treatments.

Temperature	Treatment†	
	CC‡	CC-PBC
	Cumulative nitrate-N removed	
°C	%	
Avg.§	44.5B¶	53.7A
15.5	72.8 (2.6)	86.3 (4.5)
1.5	16.2 (1.9)	21.1 (1.3)
	NRR#	
	g N m^{-3} d^{-1}	
15.5	34.9 (0.8)B	40.2 (1.9)A
1.5	7.4 (0.8)A	9.7 (0.7)A
	NRR	NRR_{CC-PBC}††
	g N m^{-3} d^{-1}	
15.5	34.9 (0.8)A	25.8 (1.2)B
1.5	7.4 (0.8)A	6.3 (0.4)A
	Temperature sensitivity	
	Q_{10}‡‡	
	3.4	3.0
	Net cumulative TC load	
	g column^{-1}	
15.5	23.7 (4.9)A	21.4 (2.9)A
1.5	3.9 (0.6)A	4.9 (0.7)A
	Net cumulative TOC load	
	g column^{-1}	
15.5	15.7 (4.8)A	12.5 (4.3)A
1.5	1.8 (0.4)A	2.0 (0.4)A
	cpN_2O production	
	g N m^{-3}	
15.5	17.2 (5.4)aA	4.5 (1.6)aB
1.5	11.8 (3.2)aA	13.9 (2.5)aA
	rN_2O	
	%	
15.5	0.5 (0.2)A	0.1 (0.05)A
1.5	1.7 (0.6)A	1.4 (0.3)A

	Position	*nosZ* (clade 1) abundance	
		log_{10} gene copy g^{-1} material	
Avg.	Inlet	9.59 (0.22)bA	9.94 (0.30)aA
Avg.	Outlet	10.95 (0.82)aA	9.02 (0.38)bB

† CC, corn cobs; CC-PBC, CC in series with plastic biofilm carrier.

‡ For the CC treatment, data are from Feyereisen et al. (2016).

§ Means in this row are averaged across temperatures.

¶ Values are means (SE). Within a row, means followed by the same uppercase letter are not significantly different at $P \leq 0.10$. Within a column, means followed by the same lowercase letter are not significantly different at $P \leq 0.10$.

Nitrate-N removal rate based on CC chamber volume alone.

†† Nitrate-N removal rate based on CC and PBC chamber volumes together.

‡‡ Q_{10} is the multiplicative change in nitrate-N removal rate for every 10°C change in water temperature. The Q_{10} values were calculated with treatment means for the warm and cold runs, not by individual columns. Thus, statistical analysis of Q_{10} was not made.

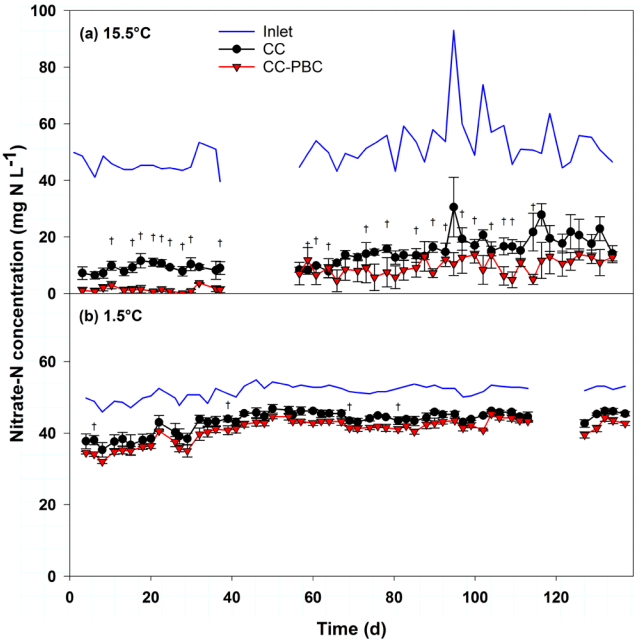

Fig. 1. Nitrate-nitrogen influent and effluent concentrations from bioreactors filled with corn cobs (CC) or CC plus plastic biofilm carrier in series (CC-PBC) at (a) 15.5°C and (b) 1.5°C. Error bars represent standard errors of the mean ($n = 3$). Days when treatments were significantly different at $P \leq 0.10$ are indicated by †.

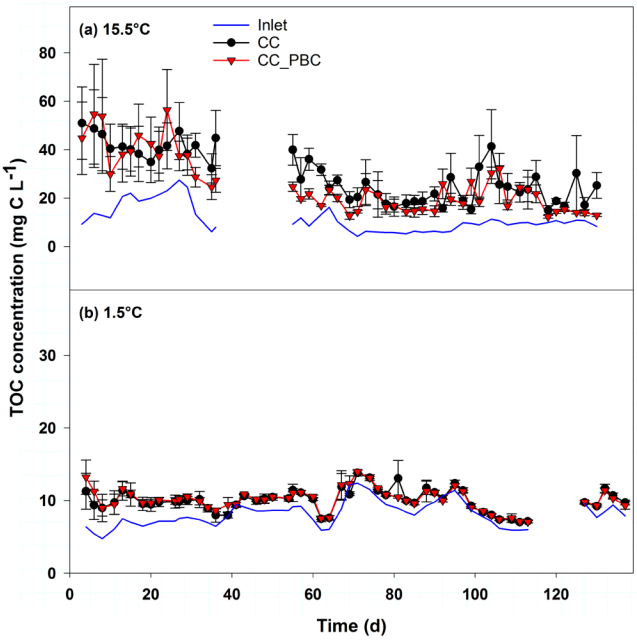

Fig. 2. Total organic carbon (TOC) influent and effluent concentrations from bioreactors filled with corn cobs (CC) or CC plus plastic biofilm carrier in series (CC-PBC) at (a) 15.5°C and (b) 1.5°C. Error bars represent standard errors of the mean ($n = 3$).

for CC and CC-PBC, respectively, and from 6.82 to 7.44 and 6.81 to 7.41 at 1.5°C for CC and CC-PBC, respectively.

Gene Abundance

Mean abundance of *nosZ* (clade 1 and clade 2) and the 16s ribosomal RNA gene was not affected by the main effects of temperature, treatment, or inlet–outlet position (Supplemental Table S8). There was a significant treatment × position interaction for the *nosZ* (clade 1) gene ($P = 0.016$, Table 1). Averaged

across temperatures, *nosZ* (clade 1) gene abundance for CC was greater at the outlet than the inlet and for CC-PBC was greater at the inlet than at the outlet. Additionally, *nosZ* (clade 1) gene abundance was greater at the outlet of the CC treatment than at the PBC outlet of the CC-PBC treatment. The two-factor analysis of gene abundance at the inlets and outlets of the CC sections of the CC and CC-PBC treatments at 1.5°C showed a significant difference for *nosZ* (clade 1) gene abundance averaged across inlets and outlets of the CC chambers ($P = 0.09$), 10.5 and 9.5 $\log_{10}$ gene copy g^{-1} substrate, for CC-PBC and CC, respectively (Supplemental Table S8). The main effect of position and the substrate × position interaction were insignificant ($P = 0.21$ and 0.82, respectively).

Dissolved Nitrous Oxide

There was no significant difference in dN$_2$O between treatments at either 1.5 or 15.5°C ($P = 0.58$ and 0.13, respectively; Supplemental Fig. S3). Across treatments, there were differences in dN$_2$O among dates at 15.5°C ($P = 0.015$, Supplemental Table S9), but not at 1.5°C ($P = 0.14$). For cpN$_2$O, the temperature × media interaction was significant ($P = 0.099$). At 15.5°C, cpN$_2$O was greater for CC than for CC-PBC; however, the difference was not significant at 1.5°C (Table 1). Across media treatments, mean rN$_2$O was significantly greater at 1.5 than 15.5°C, equivalent to 1.6 and 0.3% of cumulative nitrate-N removal, respectively.

Discussion

Across temperatures, the chamber of PBC in series after the CC media reduced nitrate-N load by 21% compared with CC alone. At 15.5°C, NRR was greater and effluent nitrate-N concentration was less for CC-PBC than for CC (Fig. 1). These indications of enhanced performance were not observed at the 1.5°C temperature. The relative contributions to the additional nitrate-N load reduction of microbial activity associated with the PBC and of the additional HRT in the PBC chamber could not be differentiated within the given experimental design.

The NRRs at 15.5°C were similar to what has been reported for CC (34.6 g N m^{-3} d^{-1}; Cameron and Schipper, 2010), which is several times as high as typical values for woodchips (2–22 g N m^{-3} d^{-1}; Schipper et al., 2010). In a recent review of woodchip bioreactors, Addy et al. (2016) reported 2 of 35 NRRs over 40 g N m^{-3} d^{-1} and 29 of 35 under 15 g N m^{-3} d^{-1}. In this study, the NRRs at 1.5°C for both treatments were greater than those reported for woodchips at temperatures <7°C, ranging from 0.5 to 6.5 g N m^{-3} d^{-1} (Addy et al., 2016), suggesting that the more readily available C of CC improves removal at colder temperatures (Feyereisen et al., 2016). The temperature sensitivity of the NRR as measured by Q_{10} was nominally less for CC-PBC than for CC (3.0 vs. 3.4, respectively), as the reduction in NRR from 15.5 to 1.5°C was less for CC-PBC than for CC (Table 1). The temperature dependence of this microbiologically mediated process is substantial, and a better answer to nitrate-N removal in cold, springtime conditions is still needed.

Denitrifiers effectively colonized the PBC material (Supplemental Table S8, Supplemental Fig. S4), although *nosZ* gene copies were never greater and were sometimes less at the outlet (PBC) of the CC-PBC treatment than at the inlet (CC). The presence of denitrifiers in the PBC chamber, with the extended residence time in the chamber, supported additional denitrification; however, the modest nitrate-N removal benefit was not associated with a corresponding reduction in aqueous C. Thus, the hypothesis that PBC would lessen aqueous C loss from the system was rejected. Additional research is also needed to address the question of limiting factor for nitrate removal, given the presence of denitrifiers, C, and available nitrate-N.

The PBC chamber was effective in reducing cpN$_2$O at 15.5°C, but not at 1.5°C. As noted above, the PBC chamber also improved NRR at 15.5°C, but not at 1.5°C. Thus, conversion of nitrate to N$_2$ gas appeared to be positively affected by the PBC chamber at the warmer temperature. During the 15.5°C experiment, dN$_2$O increased over time, indicating that the denitrification process was becoming less complete, although across treatments, rN$_2$O was 0.3%. At 1.5°C, dN$_2$O remained fairly stable for both treatments, and across the treatments, rN$_2$O was 1.6%. Few studies have reported measured dN$_2$O and rN$_2$O. Warneke et al. (2011) noted an average rN$_2$O of 3.3% over a temperature range of 16 to 24°C on a denitrification bed filled with a mixture of woodchips and sawdust. Moorman et al. (2010) determined that the ratio of dN$_2$O to nitrate-N exported from a drain tile in which water flowed through denitrification walls was 0.6% from March to June 2004 in Iowa.

In terms of application in a field bioreactor, the PBC used in this study could be replaced by a porous, natural, and less expensive material at the outlet to provide additional processing capacity without contribution of C to the system. However, since C losses from woodchip bioreactors are less than for CC, it is speculated that benefits of the postbed chamber for a woodchip-based system would be less than seen in this study. Woodchip bioreactors have exhibited a release of C on initiation (Schipper et al., 2010); the results of this study suggest that a noncarbonaceous postbed chamber would not mitigate this pollution-swapping issue. Although CC (and other agricultural-based media) have shown greater N removal rates than woodchips, other considerations in using them include the greater losses of aqueous C and faster bed degradation (Greenan et al., 2006; Cameron and Schipper, 2010).

Conclusions

Including an additional chamber filled with PBC downstream from the CC bioreactor chamber provided marginal benefits in terms of nitrate-N removal but did not achieve C removal. The PBC in the additional chamber became colonized by denitrifiers and increased the percentage nitrate-N removal, but the increase in N removal did not reduce C losses to levels that approximate organic C levels found in streams. Nitrate removal rates were increased and cpN$_2$O was decreased at 15.5°C, suggesting a benefit of the PBC chamber. The addition of the PBC after the CC reaction chamber did not improve overall bioreactor performance at the lower temperature (1.5°C). Another important consideration in the use of CC as a bioreactor medium is the reduced longevity compared with woodchips. With CC-PBC, excess C was present in the PBC chamber, as well as available nitrate. Additional research is needed to identify the limiting factor for nitrate removal.

Acknowledgments

We express gratitude to Todd Schumacher, Taylor Hoffman, Elizabeth Petesch, and Noah Slocum for their tenacious assistance. We thank the Minnesota Agricultural Utilization Research Institute (AURI) and the Minnesota Corn Research and Promotion Council for partially funding this research. We also thank the two peer reviewers and associate editor whose expert comments strengthened the manuscript.

References

Addy, K., A.J. Gold, L.E. Christianson, M.B. David, L.A. Schipper, and N.A. Ratigan. 2016. Denitrifying bioreactors for nitrate removal: A meta-analysis. J. Environ. Qual. 45:873–881. doi:10.2134/jeq2015.07.0399

Andersson, S., M. Nilsson, G. Dalhammar, and G.K. Rajarao. 2008. Assessment of carrier materials for biofilm formation and denitrification. Vatten 64:201–207.

Cameron, S.G., and L.A. Schipper. 2010. Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. Ecol. Eng. 36:1588–1595. doi:10.1016/j.ecoleng.2010.03.010

Cantafio, A.W., K.D. Hagen, G.E. Lewis, T.L. Bledsoe, K.M. Nunan, and J.M. Macy. 1996. Pilot-scale selenium bioremediation of San Joaquin drainage water with *Thauera selenatis*. Appl. Environ. Microbiol. 62:3298–3303.

Elgood, Z., W.D. Robertson, S.L. Schiff, and R. Elgood. 2010. Nitrate removal and greenhouse gas production in a stream-bed denitrifying bioreactor. Ecol. Eng. 36:1575–1580. doi:10.1016/j.ecoleng.2010.03.011

Feyereisen, G.W., T.B. Moorman, L.E. Christianson, R.T. Venterea, J.A. Coulter, and U.W. Tschirner. 2016. Performance of agricultural residue media in laboratory denitrifying bioreactors at low temperatures. J. Environ. Qual. 45:779–787. doi:10.2134/jeq2015.07.0407

Greenan, C.M., T.B. Moorman, T.C. Kaspar, T.B. Parkin, and D.B. Jaynes. 2006. Comparing carbon substrates for denitrification of subsurface drainage water. J. Environ. Qual. 35:824–829. doi:10.2134/jeq2005.0247

Greenan, C.M., T.B. Moorman, T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2009. Denitrification in wood chip bioreactors at different water flows. J. Environ. Qual. 38:1664–1671. doi:10.2134/jeq2008.0413

Ibrahim, T.G., A. Goutelle, M.G. Healy, R. Brennan, P. Tuohy, J. Humphreys et al. 2015. Mixed agricultural pollutant mitigation using woodchip/pea gravel and woodchip/zeolite permeable reactive interceptors. Water Air Soil Pollut. 226:51. doi:10.1007/s11270-015-2335-4

Moorman, T.B., T.B. Parkin, T.C. Kaspar, and D.B. Jaynes. 2010. Denitrification activity, wood loss, and N_2O emissions over 9 years from a wood chip bioreactor. Ecol. Eng. 36:1567–1574. doi:10.1016/j.ecoleng.2010.03.012

Robertson, W.D. 2010. Nitrate removal rates in woodchip media of varying age. Ecol. Eng. 36:1581–1587. doi:10.1016/j.ecoleng.2010.01.008

Saliling, W.J.B., P.W. Westerman, and T.M. Losordo. 2007. Wood chips and wheat straw as alternative biofilter media for denitrification reactors treating aquaculture and other wastewaters with high nitrate concentrations. Aquacult. Eng. 37:222–233. doi:10.1016/j.aquaeng.2007.06.003

Sander, R. 2015. Compilation of Henry's law constants (version 4.0) for water as solvent. Atmos. Chem. Phys. 15:4399–4981. doi:10.5194/acp-15-4399-2015

SAS Institute. 2011. The SAS system for Windows. Version 9.3. SAS Inst., Cary, NC.

Schipper, L.A., W.D. Robertson, A.J. Gold, D.B. Jaynes, and S.C. Cameron. 2010. Denitrifying bioreactors: An approach for reducing nitrate loads to receiving waters. Ecol. Eng. 36:1532–1543. doi:10.1016/j.ecoleng.2010.04.008

Stanley, E.H., S.M. Powers, N.R. Lottic, I. Buffam, and J.T. Crawford. 2012. Contemporary changes in dissolved organic carbon (DOC) in human-dominated rivers: Is there a role for DOC management? Freshwater Biol. 57:26–42.

Tchobanoglous, G., F.L. Burton, and H.D. Stensel. 2003. Wastewater engineering: Treatment and reuse. 4th ed. McGraw-Hill, London.

van Driel, P.W., W.D. Robertson, and L.C. Merkley. 2006. Upflow reactors for riparian zone denitrification. J. Environ. Qual. 35:412–420. doi:10.2134/jeq2005.0027

Venterea, R.T., M.S. Dolan, and T.E. Ochsner. 2010. Urea decreases nitrous oxide emissions compared with anhydrous ammonia in a Minnesota corn cropping system. Soil Sci. Soc. Am. J. 74:407–418. doi:10.2136/sssaj2009.0078

Warneke, S., L.A. Schipper, D.A. Bruesewitz, I. McDonald, and S. Cameron. 2011. Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. Ecol. Eng. 37:511–522. doi:10.1016/j.ecoleng.2010.12.006

Welander, U., and B. Mattiasson. 2003. Denitrification at low temperatures using a suspended carrier biofilm process. Water Res. 37:2394–2398. doi:10.1016/S0043-1354(03)00019-8